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Brain levels of CDK5 activator p25 are not increased in Alzheimer's or other neurodegenerative diseases with neurofibrillary tangles

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Abstract

Elevated levels of p25 and constitutive activation of CDK5 have been observed in AD brains. This has led to the hypothesis that increased p25 levels could promote neurofibrillary tangles (NFT) through CDK5-mediated hyperphosphorylation of tau, the principal component of NFTs. We examined p25 immunoreactivity in brains from sporadic and familial AD cases, as well as other neurologic diseases that exhibit NFT, such as Down's syndrome (DS), Pick's disease (Pick), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), frontotemporal dementia (FTD). Neither the p25 immunoreactivity nor the p25/p35 ratio was elevated in the AD brains or in the other tauopathies ($n = 34$) compared with controls ($n = 11$). Although Aβ peptides have been suggested to activate calpain-mediated cleavage of p35 to p25 in cultured neurons, p25 levels in brains of TgCRND8 mice, which express high levels of brain Aβ peptides, were similar to those of non-Tg littermates. Our data suggest that high Aβ levels in brain do not activate p35 proteolysis, and p25 is unlikely to be a causative agent for NFT formation in AD or other tauopathies.

Keywords: β-amyloid, neurofibrillary tangles, tau.

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Cyclin-dependent kinase 5 (CDK5) is the catalytic component of a serine/threonine kinase that is activated by neuron-specific p35, by the p35-proteolytic derivative p25, and by a homologous protein p39 (Lew *et al*. 1994; Tsai *et al*. 1994; Tang *et al*. 1995; Delalle *et al*. 1997; Honjyo *et al*. 1999; Tseng *et al*. 2002). The CDK5/p35 complex is essential during development for neuronal migration and for the correct laminar organization of the cerebral cortex (Ohshima *et al*. 1996; Chae *et al*. 1997; Ohshima *et al*. 2001; Tanaka *et al*. 2001). In adult neurons, CDK5 also participates in dopamine receptor signaling (Bibb *et al*. 1999; Bibb *et al*. 2001a; Bibb *et al*. 2001b). In contrast, the physiological role of p25 on CDK5 kinase activity remains controversial. The low turnover of artificially expressed p25 prolongs CDK5 activation and alters its subcellular localization (Patrick *et al*. 1998; Patrick *et al*. 1999), prompting speculation that endogenous p25 may target CDK5 kinase activity to incorrect compartments. CDK5 has been shown to associate with microtubules and this proximity to the microtubule-binding protein tau implicates CDK5 as a candidate tau kinase (Ishiguro *et al*. 1993; Kobayashi *et al*. 1993; Flaherty *et al*. 2000; Veeranna *et al*. 2000). Phosphorylation of tau

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Abbreviations used: AD, Alzheimer's disease; ApoE, apolipoprotein E; CBD, corticobasal degeneration; DTT, dithiothreitol; DS, Down's syndrome; FAD, familial AD; FTD, frontotemporal dementia; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HD, Huntington's disease; NFT, neurofibrillary tangles; PD, Parkinson's disease; PHF, paired helical filament; Pick, Pick's disease; PSP, progressive supranuclear palsy; SDS, sodium dodecyl sulfate; Tg, transgenic.

can reduce its interaction with microtubules and hyperphosphorylated tau is the main component of neurofibrillary tangles (NFT), one of the key neuropathological features of Alzheimer's disease (AD) (Lu and Wood 1993; Drewes *et al*. 1995; Buee *et al*. 2000). A potential relationship between CDK5/p25 and NFT pathology in AD has been proposed based on elevated p25 levels detected in AD brains (Patrick *et al*. 1999; Tseng *et al*. 2002). *In vitro* analyses support this notion because high expression of p25, but not p35, in cultured primary mouse neurons increases tau phosphorylation and disrupts the cytoskeleton (Patrick *et al*. 1999; Van den Haute *et al*. 2001), and purified recombinant p25/CDK5 complexes accelerate tau phosphorylation in cell-free assays (Hashiguchi *et al*. 2002). Acute exposure to Aβ peptides, or to agents causing calcium influx, can induce calpain-mediated p35 cleavage to p25 (Kusakawa *et al*. 2000; Lee *et al*. 2000; Alvarez *et al*. 2001), although this does not necessarily increase tau phosphorylation in cultured cells (Kerokoski *et al*. 2002). Conversely, Aβ toxicity can be reduced by CDK5 inhibition (Lee *et al*. 2000; Alvarez *et al*. 2001). These results have been interpreted to suggest that high Aβ levels in AD may trigger p35 to p25 conversion, leading to CDK5 activation and the consequent tau phosphorylation. However, tests of this hypothesis in transgenic mice overexpressing p25 have generated conflicting results with respect to increased tau phosphorylation (Ahlijanian *et al.* 2000; Takashima *et al*. 2001), and a failure to generate neurofibrillary tangles (Bian *et al*. 2002) and the increased p25 levels in AD brains have not been corroborated (Takashima *et al*. 2001; Taniguchi *et al*. 2001; Yoo and Lubec 2001; Borghi *et al*. 2002; Nguyen *et al*. 2002). Moreover, changes in p25 levels are unknown in other diseases characterized by the abnormal deposition of phosphorylated tau protein.

To explore the potential role of p25 in tau-related dementias, we have examined CDK5 and p25 immunoreactivity in 45 human brains and in a mouse model of AD, TgCRND8. We report that brain p25 levels were not elevated in AD or other neurodegenerative disorders, or in TgCRND8 transgenic mice.

Materials and methods

Tissue source

Frozen sections of brains from Caucasians without dementia ($n = 11$) or with neurodegenerative disorders that were diagnosed on the basis of clinical history and postmortem neuropathological hallmarks ($n = 34$) were obtained from the Canadian Brain Tissue Bank (Table 1). Each brain was qualitatively categorized into one of four groups (none, mild, moderate, severe) depending on the extent of neurofibrillary tangle pathology assessed by postmortem morphological examination. Distributions of group size, sex, and mean age ± SEM were: control ($n = 11$; eight male, three female; 71.7 ± 1.4 year), all AD cases ($n = 22$; 11 male, 11 female; 68.8 ± 3.8 year), other tauopathies ($n = 10$; seven male, three female; two Down's syndrome, two Pick's disease, two corticobasal degeneration, two progressive supranuclear palsy, two frontotemporal dementia; 63.0 ± 3.2 year), Parkinson's disease ($n = 1$; 77 year), and Huntington's disease ($n = 1$; 61 year). Apolipoprotein E genotyping was done as described previously (Saunders *et al*. 1993), using DNA extracted from brain tissue. Details on the production and characterization of TgCRND8 mice (C3H X C57 genetic background) have been published elsewhere (Janus *et al*. 2000; Chishti *et al*. 2001).

Homogenate preparation and gel electrophoresis

Frozen brain sections from humans or from mice that had been perfused with phosphate-buffered saline (with 1 mM EGTA) were homogenized in five volumes (g/mL) of a lysis buffer composed of: 50 mM Tris pH 7.4, 150 mM NaCl, 1% Triton-X100, 10% glycerol, 5 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol (DTT), and a broad spectrum protease inhibitor cocktail (Sigma) (Patrick *et al*. 1999). The lysates were cleared by centrifugation at 15 000 × *g* for 20 min and the resulting pellet was used to prepare sarkosyl-insoluble fractions as described previously (Greenberg and Davies 1990). The soluble fractions (supernatants) were equalized to 2 mg/mL with tricine sodium dodecyl sulfate (SDS) sample buffer (Novex), as determined by BCA Protein Assay (Pierce). Samples were boiled for 3 min and equal amounts of protein (40 μg) loaded onto precast 10–20% tricine-polyacrylamide gradient gels (Novex).

Western blotting

Separated proteins were transferred onto nitrocellulose (0.1 μm pore size Protran, Schleicher and Schuell) for western blotting (ECL Plus, Amersham) using the following antiserum: actin (monoclonal antibody AC-40, 1 : 1000, Sigma), glyceraldehyde-3-phosphate dehydrogenase (monoclonal antibody 6C5, 1 : 1000, Biodesign International), phosphorylated tau (monoclonal antibody PHF-1), human APP (monoclonal 6E10, 1 : 2000, Senetek Inc.), CDK5 (monoclonal antibody DC-17, 1 : 1000, Santa Cruz), p35 (polyclonal antibody C-8, 1 : 1000, Santa Cruz), p25 (polyclonal antiserum C-19, 1 : 1000, Santa Cruz), polyclonal anti-p35 C-terminal antiserum (1 : 1000) kindly provided by Dr Li-Huei Tsai (Harvard), and polyclonal anti-p35 C-terminal and p25 N-terminal antiserum (1 : 1000) kindly provided by Dr Koichi Ishiguro (Mitsubishi Kasei Institute of Life Sciences). Band intensities were quantified with a Storm 860 fluorescent imager (Molecular Dynamics), which assigned arbitrary values to 100-μm square pixels relative to background fluorescence to using ImageQuant software from Molecular Dynamics. Statistical comparisons between control and disease brains were done with GraphPad InStat software using two-tailed non-parametric test (Mann–Whitney *U*-test) for comparisons between two groups or a non-parametric ANOVA (Kruskal–Wallis test) for multiple comparisons.

Results

CDK5, p35, and p25 in tauopathies and in AD

To investigate a possible role for CDK5/p25 in the development of NFT, we examined CDK5, p35, and p25 levels in

Table 1 Details of frozen sections of brains from Caucasians without dementia ($n = 11$) or with neurodegenerative disorders that were diagnosed on the basis of clinical history and postmortem neuropathological hallmarks ($n = 34$) obtained from the Canadian Brain Tissue Bank

Brain	Sex	Age (years)	Condition	Postmortem delay (h)	Brain weight (g)	ApoE	NFT
1	M	70	Control	1.25	1216	3, 3	None
2	M	69		3.5	NA	3, 4	None
3	M	94	AD	12.5	1150	4, 4	Severe
4	M	75		3.25	1167	3, 4	Severe
5	F	NA	FAD	NA	NA	3, 3	Severe
6	F	NA		NA	NA	3, 4	Severe
7	F	57	DS	6	760	3, 3	Severe
8	F	51		19	750	4, 4	Severe
9	F	77	PD	11	1310	3, 3	Mild
10	F	61	HD	3	HB: 520	3, 3	Moderate
11	M	72	PICK	10	1050	3, 3	Moderate
12	M	65		12	HB: 380	3, 3	Mild
13	F	76	CBD	3.5	1130	3, 2	Mild
14	M	56		NA	HB: 675	3, 2	Mild
15	M	68	PSP	19	NA	3, 3	Severe
16	M	79		30	HB: 640	3, 3	Moderate
17	M	47	FTD	16	1490	3, 3	Mild
18	M	62		21	HB: 350	3, 2	Mild
19	M	41		4	1580	3, 4	None
20	F	70		< 10	NA	3, 3	None
21	M	60	Control	8	1400	3, 2	None
22	F	77		4.5	1390	3, 4	None
23	F	73	Age 71.7	2	NA	3, 3	None
24	M	92	SEM 1.4	6	1170	3, 3	None
25	M	74		6	NA	3, 3	None
26	M	89		5.5	NA	3, 3	None
27	M	74		12	NA	3, 3	None
28	F	49		NA	NA	3, 4	Severe
29	F	49		NA	NA	4, 4	Severe
30	M	47	FAD	12	1200	3, 3	Severe
31	F	57		12.5	1028	3, 4	Severe
32	M	49	Age 58.1	NA	NA	3, 3	Severe
33	M	64	SEM 1.3	2	NA	3, 3	Severe
34	M	73		< 24	HB: 635	NA	Severe
35	F	60		3	900	3, 3	Severe
36	F	75		3.5	970	3, 4	Severe
37	M	83		18.5	1120	3, 3	Moderate
38	M	81		16.5	1300	3, 3	Mild
39	M	NA	AD	NA	NA	NA	Severe
40	M	40		17.5	1218	3, 3	Severe
41	F	75	Age 78.5	11	970	3, 4	Severe
42	F	87	SEM 1.6	5	1140	3, 3	Severe
43	F	80		17	1400	3, 4	Severe
44	M	83		15	NA	3, 3	Moderate
45	F	87		2	NA	4, 4	Severe

AD, Alzheimer's disease; ApoE, apolipoprotein E; CBD, corticobasal degeneration; DS, Down's syndrome; FAD, familial AD; FTD, frontotemporal dementia; HB, hemibrain; HD, Huntington's disease; PD, Parkinson's disease; Pick, Pick's disease; PSP, progressive supranuclear palsy; NA, not available.

homogenates of frontal cortices from eight non-neurologic controls and 14 tauopathy cases (four AD, two DS, two Pick, two CBD, two PSP and two FTD (Table 1). Considerable variation was observed within both groups, but there were no statistical differences in the levels of soluble p25 ($p > 0.36$), p35 ($p > 0.26$), CDK5 ($p > 0.81$) when immunoreactivities were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Fig. 1a,b). As in previous reports (Patrick *et al*. 1999; Takashima *et al*. 2001), p35 migrated as a doublet in some samples, possibly due to differential phosphorylation (Saito *et al*. 2003). However, the p25/p35 ratio was not significantly altered between control and tauopathy brains [2.5 ± 0.7 (range: 0.1–6.2) and 2.3 ± 0.5 (range: 0.3–5.1), respectively]. Furthermore, in contrast to a previous report (Patrick *et al*. 1999; Tseng *et al*. 2002), it was noticeable that six out of eight control brains had substantial levels of p25.

To assess whether there is evidence for altered CDK5, p35, and p25 levels in specific subtypes of AD, we measured expression of these proteins in 11 control, 13 familial (FAD) and 11 sporadic AD (SpAD) brains (Fig. 2a, soluble). Relative to the control brains, soluble CDK5 immunoreactivity in the FAD brains was similar (95%; $p > 0.2$), but was reduced (49%; $p < 0.001$) in the SpAD brains (Fig. 2b). Three anti-p35 polyclonal antisera, which were comparable in their detection of p25 and p35, showed that levels of p25

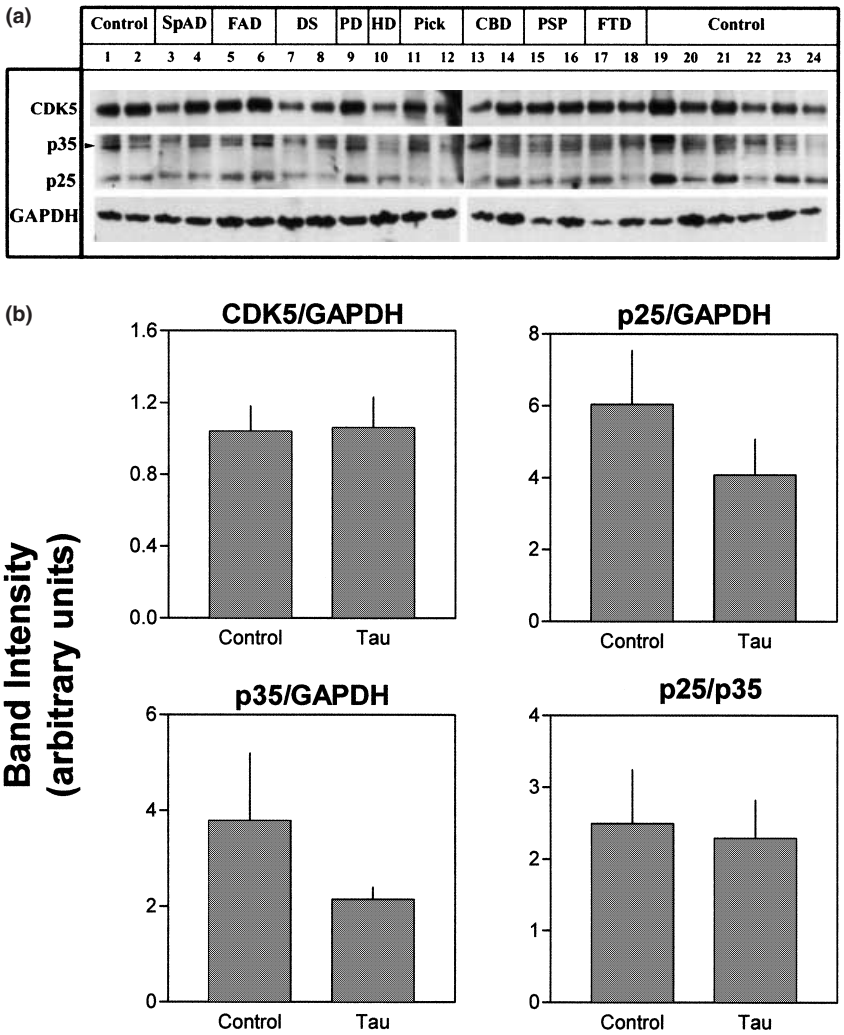

Fig. 1 CDK5, p35, and p25 immunoreactivity in tauopathies. Frontal cortices from non-neurological controls and diseased human brains were homogenized and lysates containing 20 μg of protein were separated on 10–20% Tricine gradient gels. (a) Following transfer to nitrocellulose, immunoreactivity to CDK5 (DC-17 monoclonal antibody 1 : 1000), p35 and p25 (C19, polyclonal antibody, 1 : 1000), and GAPDH (6C5, monoclonal antibody, 1 : 1000) was determined by western blotting. (b) Bars represent means and SEM of immunoreactive band intensities of CDK5, p25, p35, normalized with the GAPDH band and p25/p35 ratio from Control ($n = 8$), and tauopathy brains (Tau: AD, FAD, DS, Pick, CBD, PSP, and FTD; $n = 14$).

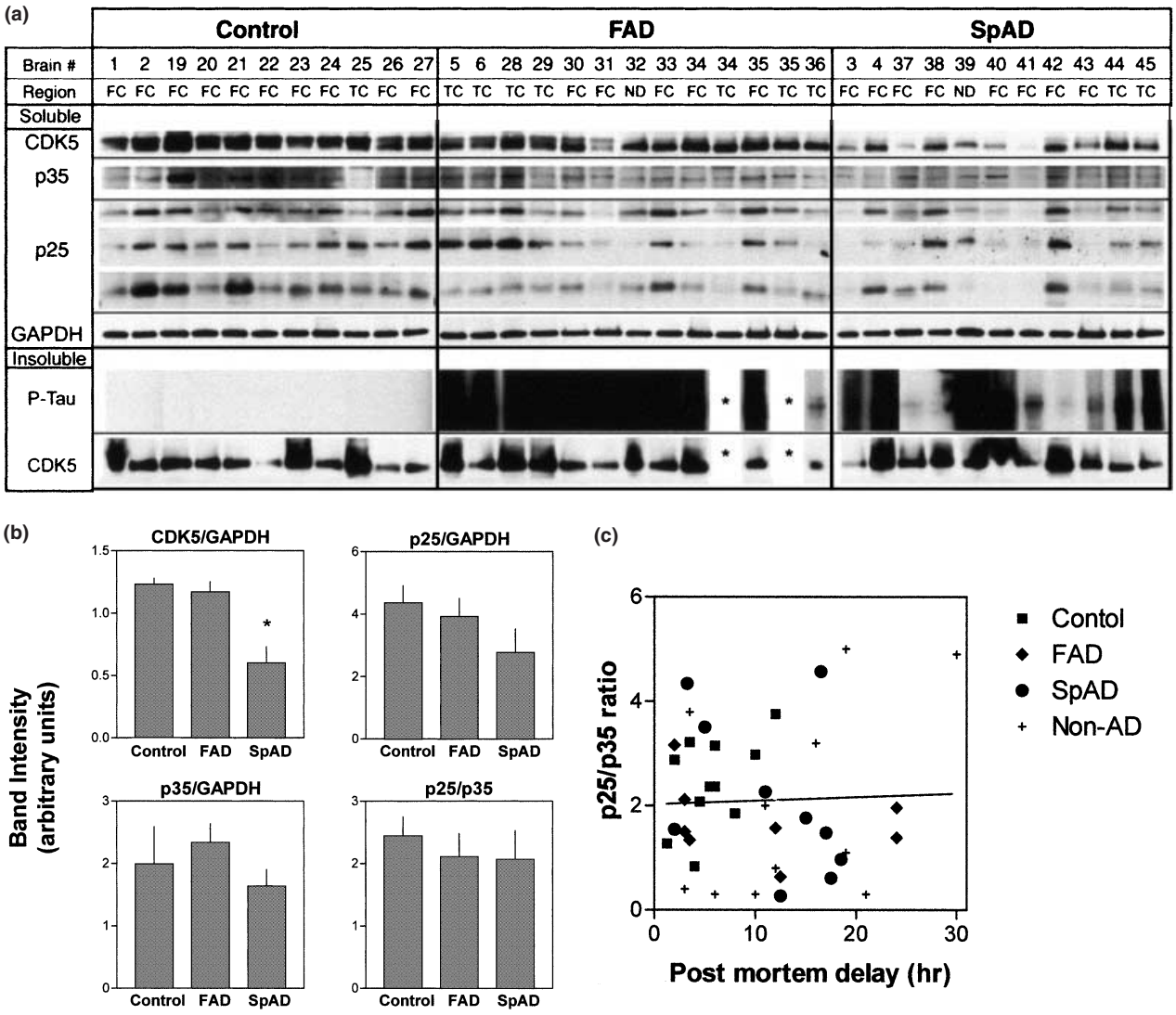

Fig. 2 CDK5, p35, and p25 levels in control, FAD, and AD. (a) Cortex from control ($n = 11$), FAD ($n = 13$), or SpAD ($n = 11$) brains was separated into detergent soluble and insoluble fractions. Soluble fractions were probed for CDK5 (DC-17 monoclonal antibody 1 : 1000, Santa Cruz), p35 (anti-p35 C-terminal polyclonal antiserum, 1 : 1000 provided by Koichi Ishiguro), and p25 (top row: C19, anti-p35 C-terminal polyclonal antiserum, 1 : 1000, Santa Cruz; middle row: anti-p35 C-terminal antiserum provided by L.H. Tsai; bottom row: anti-p35 C-terminal polyclonal antiserum, 1 : 1000 provided by Koichi Ishiguro), GAPDH (6C5 1 : 1000, monoclonal antibody). Insoluble fractions were examined for phosphorylated tau immunoreactivity (PHF-1) and CDK5. FC, frontal cortex; TC, temporal cortex; ND, region of cerebral cortex not determined; *not determined. (b) Bars show the mean and SEM of CDK5, p25 and p35 band intensities (C19, anti-p35 C-terminal polyclonal antiserum, Santa Cruz), normalized with GAPDH levels, and the p25/p35 ratio in control ($n = 11$) and FAD ($n = 13$) and SpAD ($n = 11$). *$p < 0.01$ indicates that mean value is significantly different from corresponding measure in control brains (Mann–Whitney *U*-test). (c) Scatter plot of 38 brains from Table 1 comparing the p25/p35 ratio with the reported post mortem delay. Brains 5, 6, 14, 28, 29, 32, 39 were excluded from this analysis due to insufficient information.

($p > 0.64$ control versus FAD; $p > 0.06$ control versus SpAD) and p35 ($p > 0.42$ control versus FAD; $p > 0.69$ control versus SpAD) were not significantly different between control, FAD, and SpAD brains when normalized with GAPDH immunoreactivity (Fig. 2b). The ratio of p25 to p35, an indicator of p35 to p25 conversion and of the potential interaction of CDK5 with available p35 or p25, was similar between the control and both AD populations (Fig. 2c). Although, we were unable to determine with confidence sarkosyl-insoluble p35/p25 immunoreactivity, we confirmed the clear presence of sarkosyl-insoluble phosphorylated tau detected by PHF1 antibody in these FAD and SpAD cases but not in the control brains (Fig. 2a, insoluble). Significant levels of CDK5 were recovered in the sarkosyl-insoluble fraction in all control and neurological samples; although levels were quite variable, there were no differences

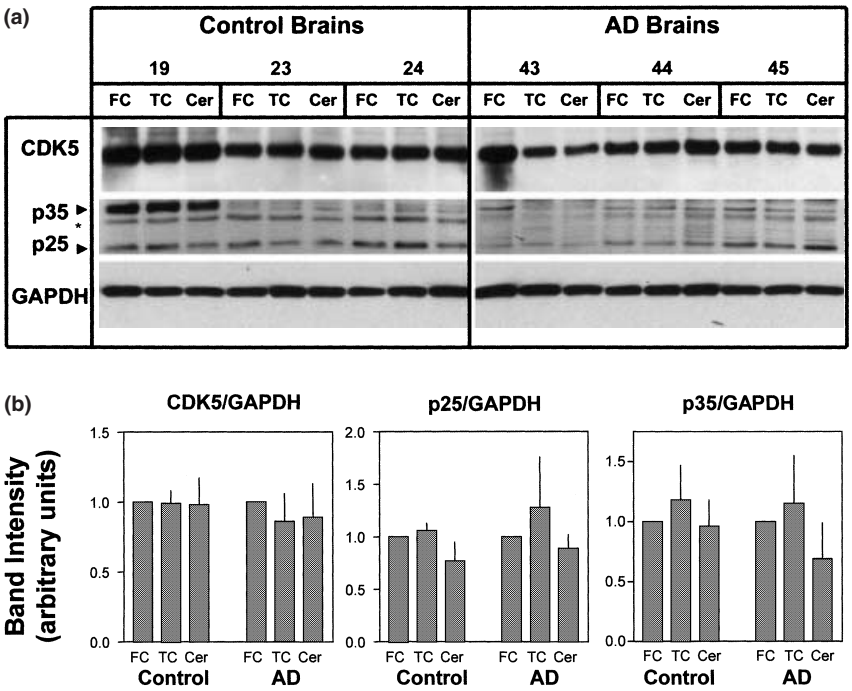

Fig. 3 CDK5 and p25 levels in frontal and temporal cortex, and cerebellum from control and AD brains. Tissue from frontal and temporal cortex, and cerebellum was dissected from frozen brains from control brains ($n = 3$) and AD ($n = 3$) brains and prepared for western blotting. (a) Immunoreactivity was determined with CDK5 (DC-17 monoclonal antibody 1 : 1000, Santa Cruz), p35 and p25 (C19, polyclonal antiserum, 1 : 1000, Santa Cruz), and GAPDH (6C5 monoclonal antibody). (b) Immunoreactive bands of CDK5, p35 and p25 from control and AD brains were quantified and normalized with the GAPDH band and are presented as a fraction of the frontal cortex signal. Brain region: FC, frontal cortex; TC, temporal cortex; Cer, cerebellum; *non-specific band.

between control and AD groups. Moreover, there was no obvious relationship between levels of insoluble CDK5 and PHF tau.

Tsai and colleagues have proposed that p35 proteolysis can occur as a postmortem artifact, and that p25/p35 ratios may be sensitive to postmortem delay (Patrick *et al*. 2001; Tseng *et al*. 2002). To investigate this, we plotted the p25/p35 ratio for the brains described in Table 1 versus the corresponding postmortem delay. No correlation was apparent ($p = 0.75$; Fig. 2d). Furthermore, the postmortem interval between groups was not different ($p = 0.25$; Kruskal–Wallis non-parametric ANOVA).

We also considered a possible link between p25 levels and apolipoprotein E (ApoE) genotype, because carriers of the ApoE ϵ4 allele are at greater risk for AD (Saunders *et al*. 1993) and increased isoform-specific binding of ApoE ϵ4 with tau potentially implicates ApoE ϵ4 in NFT formation (Huang *et al*. 1994; Strittmatter *et al*. 1994; Huang *et al*. 1995). There was no relationship between p25 levels and the APOE ϵ4 allele (Table 1). The p25/p35 ratio in the 14 patients with at least one ϵ4 allele (1.7 ± 0.2) was not significantly different from that in individuals with no ϵ4 allele (2.4 ± 0.3).

To explore the possibility of anatomical variations in p25 levels, we examined CDK5, p35, and p25 immunoreactivity in the frontal and temporal cortex, and the cerebellum of individuals with ($n = 3$) and without AD ($n = 3$) (Fig. 3a). Some immunoreactive bands with intermediate mobilities can also be seen in the AD brain samples and may represent some transitional proteolytic derivatives of p35. Additional bands migrating between p35 and p25 were also observed in previous studies (Patrick *et al*. 1999; Taniguchi *et al*. 2001). For each brain, the intensity of each protein was normalized to the value in the frontal cortex to allow comparison between brain regions. Although there was considerable variation between individuals, intrasubject levels of CDK5, p25, p35 levels did not vary in relation to brain region (Fig. 3b).

CDK5, p35, and p25 in transgenic CRND8 mice

Acute exposure to Aβ peptides has been reported to induce calpain-mediated cleavage of p35 to p25 in cultured primary mouse neurons (Lee *et al*. 2000). To test whether this mechanism is recapitulated *in vivo* with chronically high Aβ levels as in AD (Lue *et al*. 1999; McLean *et al*. 1999; Wang *et al*. 1999), we examined p35 processing in a TgCRND8 transgenic mouse model of AD. Total extractable brain Aβ in these mice, which express a double mutant form of human βAPP (KM670/671NL + V717F), increases dramatically from birth and $A\beta_{42}$ levels surpass $A\beta_{40}$ by 8 weeks (Janus

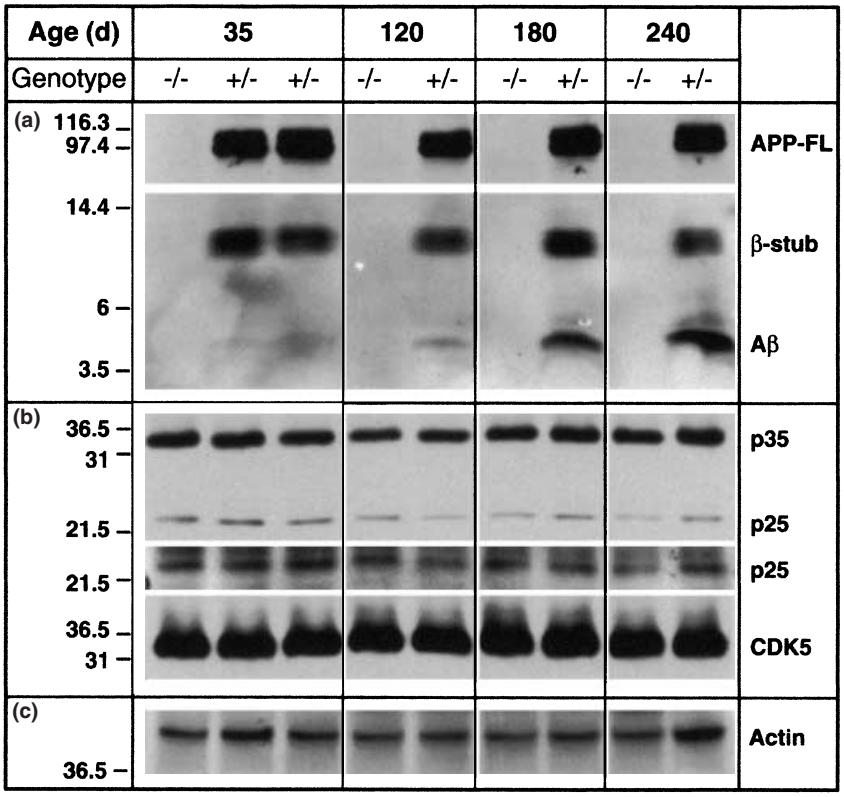

Fig. 4 Age-related expression of CDK5, p35, and p25 in TgCRND8 mice. Western blots of brain homogenates (40 μg protein/lane) from TgCRND8 mice (+/–) and non-Tg (–/–) littermates at ages (days): 35, 120, 180, and 240. (a) Full-length βAPP (APP-FL), β-secretase cleavage product (β-stub), and Aβ peptides (Aβ) were detected with human βAPP-specific monoclonal antibody 6E10 (1: 2000, Senetek). (b) Top panel: p35 and p25 detected by C-19 polyclonal antiserum (1 : 1000, Santa Cruz). Identical results were obtained with anti-p35 C-terminal antiserum provided by L.H. Tsai and Koichi Ishiguro (not shown). Middle panel: p25 detected by anti-p25 N-terminal polyclonal antiserum supplied by Koichi Ishiguro. Bottom panel: CDK5 detected by DC-17 monoclonal antibody (1 : 1000, Santa Cruz). (c) Actin detected by monoclonal antibody AC-40 (1 : 1000, Sigma).

et al. 2000; Chishti *et al*. 2001). Figure 4(a) shows the unchanging expression of full-length human APP (APP-FL) in hemizygous TgCRND8 mice from 5 weeks to 8 months of age and an age-related rise in Aβ peptides, as compared with their non-Tg littermates. p35, p25, and CDK5 immunoreactivity did not vary in either the Tg or non-Tg animals, or with increasing age and Aβ levels (Fig. 4b). Identical results were obtained using two other anti-p35 polyclonal antisera and with six additional TgCRND8 animals aged 2–12 months (results not shown).

Discussion

In the present study, we examined whether p35 processing is altered in AD or other neurodegenerative diseases exhibiting pathological accumulations of phosphorylated tau in CNS neurons, including DS, Pick, CBD, PSP, and FTD. There was no correlation between p25 levels and the presence of NFT using three different polyclonal anti-p35 antisera. Furthermore, in contrast to previous reports (Patrick *et al*. 1999; Tseng *et al*. 2002), there was no elevation of p25 levels even in the subset of brains with pure AD. In fact, p25 immunoreactivity tended to be lower in the AD brains when normalized to GAPDH levels, although the change did not reach statistical significance. Moreover, we did not detect substantial regional differences in individual brains. Interestingly, we found that detergent extractable CDK5 levels were reduced in sporadic AD brains relative to control and FAD brains, whereas insoluble CDK5 immunoreactivity was preserved. The functional implications of this change are unclear, because neither the soluble nor insoluble CDK5 levels correlated with phosphorylated tau. However, the absence of change in the insoluble CDK5 levels in the AD brains is consistent with the lack of change in p25 levels or the p25/p35 ratio, because interaction of p25 with CDK5 would be expected to reduce the amount of CDK5 complex associated with the particulate fraction (Patrick *et al*. 1999; Kusakawa *et al*. 2000).

Our results differ from the findings of Tsai and colleagues (Patrick *et al*. 1999) who initially reported barely detectable

p25 immunoreactivity in control brains, but a 20–40-fold increase in seven out of eight AD brains. Their subsequent analysis with larger sample size yielded mean p25/p35 ratios that were approximately 1.7 and 5 for control and AD brains, respectively (Tseng *et al*. 2002); however, there was considerable overlap of p25/p35 ratios for the majority of the AD and control brains, ranging from 1.3 to 8. In our samples, the average p25/p35 ratio was 2.4 (range = 0.8–3.8) and 2.1 (range = 0.3–5.7) for the control and AD cases, respectively. The reasons for the disparity in the AD p25/p35 ratios between studies are not immediately apparent. It is unlikely that the p35 to p25 conversion in our samples occurred during tissue preparation, because we have used the same protocol used by Tsai and colleagues (Patrick *et al*. 1999). The argument that prolonged postmortem delays expose p35 to proteases that are activated in dying neurons (Patrick *et al*. 2001; Taniguchi *et al*. 2001) does not account for the different results because the mean postmortem interval for the control brains used in our study (5.7 ± 1.0 h) was substantially less than the 21 ± 1.7 h reported by Patrick *et al*. (1999), but similar to that reported by Tseng *et al*. (2002) (∼3 h). Indeed, the p25/p35 ratios in the present study are comparable to the control values obtained in the Tseng *et al*. (2002) analysis. Moreover, we observed no correlation between the p25/p35 ratio and the corresponding postmortem delay. Our results are more compatible with other reports showing control human brains expressing p25 in excess of p35, and no difference in the p25/p35 ratio relative to AD brains (Takashima *et al*. 2001; Taniguchi *et al*. 2001; Van den Haute *et al*. 2001; Yoo and Lubec 2001).

The neurotoxicity following acute Aβ peptide treatment of cultured primary mouse neurons has been proposed to involve calpain-mediated proteolysis of p35 to p25 (Lee *et al*. 2000; Li *et al*. 2003). The p25 derivative is more stable than its parent peptide when expressed artificially, and can sequester CDK5 in the cell soma and up-regulate its kinase activity (Patrick *et al*. 1998; Patrick *et al*. 1999; Ahlijanian *et al*. 2000). We have explored whether this cascade can be extended to an *in vivo* setting using TgCRND8 mice, which express a double mutant form of βAPP. TgCRND8 mice accumulate Aβ in the brain, which is accompanied by Aβ plaques and severe cognitive deficits at three months of age (Janus *et al*. 2000; Chishti *et al*. 2001). Despite the significant age-related rise in Aβ peptide levels (especially of $A\beta_{1-42}$) between 1 and 8 months, there was no concomitant change in p35 or p25 levels. This suggests considerable divergence between the cell culture and *in vivo* models of Aβ toxicity with respect to p35 metabolism. While this result could be dismissed because of the absence of NFT in this and other Tg mice overexpressing Aβ, it is worth noting that the acute toxicity studies showing Aβ-induced calpain activation and p35 cleavage were also performed on murine neurons. There also appear to be important differences in p35 metabolism in mouse and human neurons; comparison of p25/p35 ratios, presented in this study and in previous reports (Lee *et al*. 2000; Taniguchi *et al*. 2001; Van den Haute *et al*. 2001; Bian *et al*. 2002; Tseng *et al*. 2002), suggests that murine cells have more p35 than p25 (p25/p35 ratio < 1), whereas human cells contain more p25 than p35 (p25/p35 ratio > 2). Whether this is strictly a species-related phenomenon or whether it reflects some early postmortem changes is currently unclear, and needs to be resolved. On balance, the TgCRND8 mouse model is a more representative model of Aβ biology in AD patients than acute studies with cultured neurons. Furthermore, the absence of increased p25 levels in the transgenic mouse model is consistent with the lack of p25 accumulation in our collection of AD brains. When taken together, our results suggest that cleavage of p35 to p25 is unlikely to contribute significantly to AD pathology.

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